Claim Rejections - 35 U.S.C. § 103

Claims 1, 2, 4-6, 9-17, 21-45, 46-47 and 51-55 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Bernstein (cited previously) in view of Beaumont (US Patent 5,264,372), Krikorian, Brewer and any one of Zhang, Myer, Nakagawa, Levine, Nagy, Nakamaki or Liu. The examiner asserts that the combination of Bernstein's obtaining shortening of mRNA molecules with poly(A) tracts using a cytoplasmic extract with a ribosomal salt wash with Beaumont's effectively separating components in a biological sample by centrifugation at 100,000 x g, and further with Krikorian's in-vitro degradation system which uses a cell lysate of HeLa cells, or Brewer's system for monitoring RNA turnover, in combination with the latter documents which demonstrate binding patterns of various RNA-binding proteins and their roles in RNA stability.

Applicants respectfully request reconsideration of the rejection. Applicants submit that while other investigators in the field of mRNA turnover included various cellular components in their *in-vitro* systems and obtained what they considered to be mRNA turnover, Applicants simply used the supernatant from a 100,000 x g cellular extract, discarding all other cellular components, and obtained regulated mRNA deadenylation and degradation. Bernstein and colleagues discarded the 100,000 x g supernatant and isolated and used only polysomes or materials extracted from polysomes using high salt. **They discarded what Applicants used, and *vice versa*.** Applicants surprisingly and unexpectedly obtained a system that successfully recapitulates mRNA turnover without whole-cell components, polysomes, polysome extracts, etc., *i.e.*, ***less*** than any prior-art investigator used. For this reason, Applicants believe their claims are unobvious over the combination of the prior art cited by the examiner.

Specifically, Bernstein **discarded** Applicants' 100,000 x g supernatant and processed the isolated polysome material instead, extracting them with high salt and using this extract, "RSW", in their system. Thus, Applicants contend that recapitulating mRNA turnover in the absence of

RSW is unexpected and unobvious.

Moreover, Bernstein et al. do not address the mechanism of poly(A) removal and subsequent degradation in their system other than to note that the accumulation of deadenylated RNA intermediates is consistent with a decay pathway in which deadenylation precedes degradation of the RNA body. Their results are consistent with one of three biochemically distinct pathways, of which only the third characterizes the observed *in-vivo* mechanism (references mentioned further below):

1. non-specific 3'-5' exonucleolytic degradation that would indiscriminately remove both poly(A) and upstream non-adenylated sequences;

2. an endonucleolytic cleavage event at a discrete site within the 3' untranslated region which would remove the poly(A) tail as a single unit followed by 3' exonucleolytic degradation of the RNA body; and

3. specific exonucleolytic removal of the poly(A) tail by a cap-dependent deadenylase followed by degradation of the RNA body by either a 5'-3' or 3'-5' exonucleolytic enzyme.

Subsequent analyses show that activities relevant to mechanisms 1 and 2 are present in the polysome system used by Bernstein et al. Pathway 1: Caruccio and Ross [*J Biol Chem* **269**: 31814-31821 (1994); copy enclosed] purified to apparent biochemical homogeneity a 33 kDa 3' - 5' exonuclease present in the polysomal RSW fraction that was not poly(A) specific. Pathway 2: Lee et al. (*J Biol Chem* **273**: 25261-25271; 1998; copy enclosed) purified to apparent biochemical homogeneity an endonuclease present in the polysomal RSW fraction from rat liver cells. This endonuclease cleaves at specific sequences in the 3' UTR of the c-myc mRNA. The activity of this enzyme, therefore, removes the poly(A) tail in one step, and hence it is not a deadenylase and acts as a random RNA exonuclease.

The relevance of these activities to *bona-fide* regulated *in-vivo* mRNA turnover is unclear at the present time (see, for review, Wilusz CJ et al., *Nat Rev Mol Cell Biol* **2**:237-46 (2001); copy enclosed). In contrast, Applicants' system proceeds by pathway 3 which appears by all current accounts to accurately reflect the *in-vivo* mechanism of turnover (see review). Therefore,

Applicants' system proceeds by a mechanism that was neither anticipated or biochemically substantiated in polysome extracts.

Applicants' respectfully disagree with the examiner's assertion that Beaumont provides any connection between Bernstein's discarding the 100,000 x g supernatant and it being an obvious selection for a successful mRNA turnover recapitulating system. At the time of Applicants' work, and as evidenced by the prior art cited by the examiner, it was believed that polysomes contained the critical components for mRNA turnover, hence the focus of prior investigators on using polysomes or polysomal components (and discarding the 100,000 x g supernatant) to attempt to mimic *in-vivo* mRNA turnover *in vitro*.

Krikorian adds nothing more. This study utilized lysates (no centrifugation steps) of viral- or mock-infected HeLa cells. Mock-infected cells showed no significant mRNA degradation as did those infected with a mutant HSV-1 virus lacking a gene (vhs) critical for mRNA degradation. Vhs protein is a known endoribonuclease (*e.g.,* Lu et al., *J. Virol.* **175**:172-1185; 2001; abstract enclosed) that is poly(A) independent (see Karr et al., *Virology* **264**:195-204; 1999; abstract enclosed). Lysates prepared from wild-type-virus-infected cells exhibited no evidence for deadenylation (Figure 2 does not reveal any shortening of the GAPD mRNA prior to its disappearance). Notwithstanding these findings or Applicants' success in recapitulating mRNA turnover without using an extract of virally-infected cells, Krikorian did not use a simple 100,000 x g cell supernatant to achieve a system that recapitulates mRNA turnover. In summary, since HeLa lysates in the Krikorian system contain *no* mRNA degradation activity and the mRNA degradation observed in Krikorian lysates is dependent upon the HSV-1 vhs protein [a known poly(A) *independent endo* (not exo) nuclease], the study is totally unrelated to the application currently being considered which involves mechanisms and assays for the regulated deadenylation and degradation of mRNAs through endogenous cellular factors.

Brewer also adds nothing more. Brewer threw away the 100,000 x g supernatant and used isolated polysomes for the mRNA turnover studies. Thus, the systems are different, and no motivation to utilize a supernatant is provided in Brewer's studies.

Chen, Zhang, Myer, Nakagawa, Levine, Nagy, Nakamaki and Liu each identify either AU-rich elements as important regulatory sequences involved in mRNA turnover or identify proteins that bind to this element. It is important to note here that simple binding of a protein to an RNA sequence does not imply function. The literature is full of RNA binding proteins that interact in a non-functional fashion with important sequences. For example, hnRNP C protein interacts with conserved sequence elements in polyadenylation signals, but this interaction does not have a functional impact on the process of 3' end formation [see Chou et al., *Nucleic Acids Res* **22**:2525-31 (1994); copy enclosed]. In all of the cases cited above, a direct role for the identified AU-rich element binding proteins is lacking. Binding should not be confused with functional significance, especially for RNA binding proteins. Applicants believe that without the appreciation obtained by Applicants that the use of a simple 100,000 x g supernatant can recapitulate mRNA turnover, none of these citations add any motivation or render obvious Applicants' finding. No suggestion is made in any of these references that the components present in a 100,000 x g cell supernatant would be both necessary and sufficient to achieve the claimed mRNA deadenylation and degradation that recapitulates in-vivo mRNA turnover. As mentioned above, focus on whole-cell extracts or isolated polysomes or their components has been the clear focus of other investigators in this area. Moreover, the work of Levy et al. cited in the previously-submitted Information Disclosure Statement (BD, BE) also fails to meet the instant claims by not depleting the extract of proteins that bind polyadenylate. Furthermore, the assay employed by Levy et al. did not involve polyadenylated RNAs nor, therefore, deadenylation in any explicit or implied way. Applicants' system, therefore, differs very dramatically from anything performed or anticipated by the work of Levy et al.

Thus, Applicants maintain that no motivation existed prior to Applicants use of a 100,000 x g cell extract supernatant to achieve a *bona-fide* mRNA turnover system that recapitulates in-vivo mRNA deadenylation and degradation, and in light of the foregoing, withdrawal of the rejection is respectfully requested.

Fees

A check in the amount of $55.00 is enclosed for a one-month extension of time. No other fees are believed to be necessitated by the instant response. However, should this be in error, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment, or to credit any overpayments.

CONCLUSION

Applicants respectfully request entry of the foregoing amendments and remarks in the file history of the instant Application. The Claims as amended are believed to be in condition for allowance, and reconsideration and withdrawal of all of the outstanding rejections is therefore believed in order. Early and favorable action on the claims is earnestly solicited. Should a telephone discussion assist in the prosecution of the application, the Examiner is invited to call the undersigned at (201) 487-5800, ext. 103, to effect a resolution.

Respectfully submitted,

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Enclosure: Supplemental Information Disclosure Statement, PTO-1449, and copies of documents cited above